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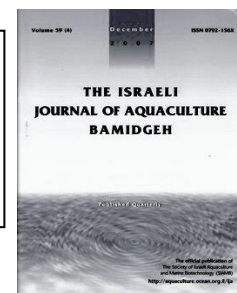
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Replacement of Fishmeal by Mixed Animal and Plant Protein Sources in Diets for Juvenile Mangrove Red Snapper (*Lutjanus argentimaculatus*) (Forsskål, 1775)

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Keywords: *Lutjanus argentimaculatus*; fishmeal; soybean meal; peanut meal; fish performance

Abstract

A feeding trial was undertaken to develop a low fishmeal practical diet for mangrove red snapper (*Lutjanus argentimaculatus*) by replacing 60% fishmeal (FM) protein with either soybean meal (SM) or peanut meal (PM), and 10% fish soluble (FS) or krill meal (KM) supplementation. Seven isonitrogenous (crude protein: 40% dry matter) and isolipidic (crude lipid: 9% dry matter) diets were prepared (FM100, FM40+SM, FM40+SM+FS, FM40+SM+KM, FM40+PM, FM40+PM+FS, FM40+PM+ KM). Triplicate groups of fish (initial body weight: 1.46 ± 0.02 g, mean \pm S.E.M) were stocked in 150L circular fiberglass tanks at a density of 18 fish per tank. Results showed no significant ($p > 0.05$) differences in final body weight (FBW) values or weight gain (WG) between fish fed the FM100 diet, and those fed FM40+SM, FM40+SM+FS and FM40+SM+KM diets. For fish fed the FM40+PM diet, WG was significantly lower when compared with fish fed the other experiment diets. Supplementation of 10% FS or KM improved growth parameters of fish fed diets containing PM. Feed efficiency (FE), protein efficiency ratio (PER) and protein productive value (PPV) showed similar tendencies to the growth indices. No significant differences in feed intake (FI) were observed among all the different dietary treatments. Significant lower whole-body protein and lipid content were recorded in fish fed FM40+PM diet. The present study indicates that 60% of fishmeal protein can be replaced by soybean meal or by a mixture of peanut meal and 10% FS or KM without affecting growth performance of *Lutjanus argentimaculatus*.

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Introduction

Mangrove red snapper (*Lutjanus argentimaculatus*) is a popular economically valuable fish species cultured in the south of China and other east Asian countries due to its excellent flesh quality and high market values (Emata and Borlongan, 2003). Previous studies showed that 43% dietary protein level was suitable for the rapid growth of Mangrove red snapper (Catacutan *et al.*, 2001; Abbas and Siddiqui, 2013). As this is a carnivorous fish species, the diet usually contains a large amount of fishmeal for dietary palatability and normal growth. Fish meal supply however is facing shortages, and the price has greatly increased since 2000 (FAO, 2014). Large quantities of fish meal inclusion in the diet have resulted in the high price of commercial feed. Therefore, it is important to assess the possibility of finding other protein sources to replace fishmeal for Mangrove red snapper feed.

Soybean meal is one of the most widely used alternative plant ingredients in aquatic feed (Lovell, 1988). It has been successfully used to partially or totally replace fishmeal in many fish species, such as striped bass (Gallagher, 1994), cobia (Zhou *et al.*, 2005), cuneate drum (Wang *et al.*, 2006), sharpnose seabream (Hernández *et al.*, 2007), and hybrid European cat fish (Havasi *et al.*, 2015) etc. Peanut meal is the fourth largest oilseed crop and is cultivated in more than 100 countries with annual global production reaching about 35.5 million tons (FAO statistics, 2015). Due to high crude protein content, peanut meal can be used in aqua feed and partially replace fishmeal (Yue *et al.*, 2012). However, plant protein sources generally have imbalanced amino acids composition, have poor palatability, and anti-nutritional factors which limit their use (Hardy, 2010). They do however have high digestibility and nutrient values, and fewer side effects when they are added into fish diets (Bureau *et al.*, 1999). Fish soluble (FS) and krill meal (KM) are natural sources for feed stimulatory substances and are often used in commercial feeds (Kader *et al.*, 2010). Studies have shown that hulled soybean meal supplemented with FS and KM could completely replace fishmeal in diets for juvenile red sea bream without any adverse effects on fish performance (Kader *et al.*, 2012).

A previous study showed that fishmeal could be partially replaced by defatted soybean meal in formulated diets for mangrove red snapper (Catacutan and Pagador, 2004). However, no information is available on the possibility of fishmeal replacement by peanut meal in Mangrove red snapper. A blend of different ingredients in appropriate proportions can achieve balanced nutritional composition, improve feed palatability, and replace fishmeal at high levels (Kader, and Koshio, 2012). Based on the information above, the object of the present study was to evaluate the effects on growth performance and feed utilization of Mangrove red snapper when replacing fishmeal with soybean meal (SM) or peanut meal (PM) alone or when combined with fish soluble or krill meal.

Materials and Methods

Seven isonitrogenous (crude protein: 40% of dry matter) and isolipidic (crude lipid: 9% of dry matter) diets which included a high fishmeal control diet and six different low-FM diets were prepared (Table 1). In this experiment, 60% replacement levels of FM protein by solvent-extracted SBM or PM, based on a previous study by Kader *et al.* (2010) showed that 60% FM protein can be replaced by plant protein feedstuffs in red sea bream diets when supplemented with FS and krill meal. All dry ingredients were well ground, weighed, and mixed in a Hobart mixer (A-200T Mixer Bench Model unit, Resell Food Equipment Ltd., Ottawa, Canada) for 30 min. Oil was gradually added while constantly mixing the dough after which 30-50 mL of water for each 100 g of dry matter was slowly blended into the mixture. The diets were formed into 1.5 mm diameter pellets using a twin-screw extruder, then air-dried and stored at -20°C until use.

Table 1. Composition of the experimental diets (% dry-matter basis).

Ingredient	Diet groups						
	FM100	FM40 +SM	FM40 +SM+FS	FM40 +SM+KM	FM40 +PM	FM40 +PM+FS	FM40 +PM+KM
Fishmeal(Anchovy) ¹	50	20	20	20	20	20	20
Soybean meal ²		45	37	37	0	0	0
Peanut meal ³	0	0	0	0	42	34	34
Fish soluble ⁴	0	0	10	0	0	10	0
Krill meal ⁵	0	0	0	10	0	0	10
CAA mixture ^a		2	0	0	2	0	0
Fish oil	2	4.5	4	4	4.5	4	4
Soybean lecithin	1	1	1	1	1	1	1
Cellulose	20.2	0.7	1.2	1.2	3.7	4.2	4.2
Others ^b	26.8	26.8	26.8	26.8	26.8	26.8	26.8
Proximate composition (%)							
Dry matter	95	95.3	94.5	92.8	93.4	95.4	93.1
Crude protein	39.2	39.9	40.9	40.8	41.5	40.8	40.1
Crude lipid	9.1	8.8	8.5	9.1	8.7	9.9	9.3
Crude ash	11.8	10.1	11	12.1	10.3	10.1	11.3

^a Crystalline amino acid mixture: 0.5% for each of lysine, methionine, taurine, glycine.

^b Others: Wheat flour 22%, Vc 0.3%, Vitamin mixture 1%, Mineral mixture 3%, Choline chloride (50%) 0.5% Vitamin mixture contained (as kg⁻¹ dry diet): retinol IU. 50 000; cholecalciferol IU. 2000; a-tocopherol IU. 300; thiamin 37; riboflavin 48; pyridoxine 20; cyanocobalamin 0.1; folic acid 10; calcium pantothenate 74; menadione 11; ascorbic acid 240; myo-inositol 337; biotin 0.5; nicotinic acid 300 (from Ibeas et al. 2000). Mineral mixture contained (as g/kg dry diet): Ca(H₂PO₄)₂·H₂O, 6.80; Ca(C₆H₁₀O₆)·3H₂O, 17.42767; FeSO₄·7H₂O, 0.25; MgSO₄·7H₂O, 6.60; K₂HPO₄, 12.00; NaH₂PO₄·H₂O, 4.40; NaCl, 2.25; AlCl₃, 0.0042; KI, 0.0075; CuSO₄·5H₂O, 0.025; MnSO₄·H₂O, 0.035; CoCl₂·H₂O, 0.05; ZnSO₄·7H₂O, 0.15; Na₂SeO₃·1.5H₂O, 0.000635.

¹ Yongsheng Feed Corporation, Binzhou, China; proximate composition (% dry matter): moisture, 7.9; crude protein, 70.9; crude lipid, 10.1.

² Yongsheng Feed Corporation, Binzhou, China; proximate composition (% dry matter): moisture, 9.1; crude protein, 50.0; crude lipid, 1.32.

³ Yongsheng Feed Corporation, Binzhou, China; proximate composition (% dry matter): moisture, 10.0; crude protein, 55.4; crude lipid, 5.76.

⁴ Yuxin Feed Corporation, Rongcheng, China; proximate composition (% dry matter): moisture, 41.3; crude protein, 37.1; crude lipid, 6.2.

⁵ Krillsea Operation AS, Fosnavåg, Norway; proximate composition (% dry matter): moisture, 41.3; crude protein, 30.5; crude lipid, 8.2.

Experimental fish

Mangrove red snapper *Lutjanus argentimaculatus* juveniles obtained from a commercial producer (Xincun Fish Farm, Linshui, Sanya, China) were reared and fed a commercial diet in indoor culture systems where they were acclimated to experimental conditions at a density of 30 fish per tank for 2 weeks. After acclimation, fish (initial body weight 1.46±0.02g) were pooled and randomly distributed into 21 fiberglass tanks, 18 fish per tank.

Experimental layout

The 8 week feeding trial was conducted in 150 L fiberglass tanks connected as a closed recirculating system with coral-sand filter (25 cm thickness) and a primary biological filter. Low-pressure electrical blowers provided aeration via air stones. Filtered water was supplied at a flow rate of 2.2 L/min per tank, and water temperature was measured daily and maintained at 27±1°C. Dissolved oxygen (>7.05 mg/L), pH (8.05-8.23), salinity (30.1-33.2 g/L), and total ammonia (<0.10 mg/NL) were measured weekly.

Six experimental diets and one control diet were randomly assigned in the 21-tank system and the dietary treatment implemented in triplicate. Fish were handfed their respective prescribed diets to satiation twice daily at 0800h and 1700h until the floating extruded pellets sank. Feed intake was recorded daily. Fish feces were siphoned out twice a day after feeding.

At the end of the trial, fish from each tank were deprived of food for 16 h before sampling and euthanized with MS-222 (10 mg/L). Four fish from each tank were randomly collected for analysis of whole-body composition. The blood of eight fish per

tank was taken from the heart with heparinized syringes, individually weighed, and the viscera, liver, intestine and intraperitoneal fat dissected. White muscle was also dissected from both sides of the fillets without skin. Plasma was separated by centrifugation at $3500 \times g$ for 10 min and stored at -80°C until analysis of total protein (TP), cholesterol (CHOL), total tricylglycerol (TG), high density lipoprotein-cholesterol (HDL-C), and low-density lipoprotein-cholesterol (LDL-C).

Crude protein ($\text{N} \times 6.25$) was determined by the Kjeldahl method after acid digestion using an auto Kjeldahl System (1030-Auto-analyzer, Tecator, Sweden). Crude lipid was determined by ether extraction using a Soxtec System HT (Soxtec System HT6, Tecator, Sweden). Dry matter was analyzed by oven-drying at 105°C for 24 h until reaching constant weight. Crude ash was determined by incinerating samples at 550°C in a muffle furnace for 24 h. Plasma TP, CHOL, TG, HDL-C and LDL-C were determined using an automatic blood analyzer (Hitachi 7170A, Hitachi, Tokyo, Japan).

The following formulae were used to compute various body condition indices:

Condition factor (CF): $100 \times (\text{live weight, g}) / (\text{body length, cm})^3$;

Hepatosomatic index (HSI): $\text{liver weight} \times 100 / \text{body weight}$;

Intraperitoneal fat (IPF) ratio: $\text{IPF weight} \times 100 / \text{body weight}$.

Fish performance was measured as:

Weight gain (WG): $100 \times (\text{final mean weight} - \text{initial mean weight}) / \text{initial mean weight}$;

Feed efficiency (FE): $(\text{final mean body weight} - \text{initial mean body weight}) / \text{Feed intake}$;

Protein efficiency ratio (PER): $\text{g gain} / \text{g protein fed}$;

Protein productive value (PPV): $100 \times \text{retained protein (g)} / \text{protein fed (g)}$.

All data are presented as means \pm S.E.M and subjected to one-way analysis of variance (ANOVA) ($n=3$) to test the effects of experimental diets using the software of SPSS (version 13.0) for Windows. Duncan's multiple range tests was used to resolve differences among treatment means. Differences among means were considered significant at $p < 0.05$.

Results

Growth performance and feed utilization of fish fed the experimental diets are shown in Table 2. Fish fed FM40+SM+FS diet, FM40+SM diet, FM40+SM+KM or FM40+PM+KM diet showed no significant differences ($P > 0.05$) in WG compared with the FM100 (control) diet. Fish fed FM40+PM diet had the lowest WG among all the treatments, with a significant difference with FM100 diet ($p < 0.05$). There were no significant differences in FI among all the experiment treatments ($P > 0.05$). Significantly lower FE, PER and PPV were observed in fish fed FM40+PM ($p < 0.05$). Survival rates of fish fed different experimental diets were not significantly different ($p > 0.05$).

Table 2. Growth performance and feed utilization of juvenile *L. argentimaculatus* fed the experimental diets for 8 weeks¹

	WG ²	FI ³	FE ⁴	PER ⁵	PPV ⁶
FM100	651 \pm 143 ^{ab}	17.88 \pm 1.09	0.51 \pm 0.10 ^a	1.31 \pm 0.26 ^a	0.24 \pm 0.05 ^a
FM40+SM	741 \pm 130 ^a	18.90 \pm 2.10	0.60 \pm 0.07 ^a	1.51 \pm 0.17 ^a	0.29 \pm 0.04 ^a
FM40+SM+FS	764 \pm 75 ^a	20.07 \pm 2.36	0.58 \pm 0.07 ^a	1.43 \pm 0.17 ^a	0.29 \pm 0.04 ^a
FM40+SM+KM	656 \pm 90 ^{ab}	16.84 \pm 1.51	0.59 \pm 0.02 ^a	1.44 \pm 0.06 ^a	0.28 \pm 0.02 ^a
FM40+PM	374 \pm 22 ^c	15.75 \pm 3.34	0.35 \pm 0.06 ^b	0.86 \pm 0.15 ^b	0.15 \pm 0.02 ^b
FM40+PM+FS	518 \pm 79 ^{bc}	16.00 \pm 3.26	0.48 \pm 0.12 ^a	1.18 \pm 0.29 ^{ab}	0.22 \pm 0.05 ^a
FM40+PM+KM	685 \pm 74 ^{ab}	17.09 \pm 3.23	0.57 \pm 0.11 ^a	1.42 \pm 0.27 ^a	0.28 \pm 0.05 ^a

¹ Values are means \pm S.E.M. of three replicates and values within the same row with different letters are significantly different ($P < 0.05$).

² WG (%) = $100 \times (\text{final mean weight} - \text{initial mean weight}) / \text{initial mean weight}$.

³ FI (g fish⁻¹): Feed intake (as dry matter basis).

⁴ FE = $(\text{FBW} - \text{IBW}) / \text{Feed intake}$.

⁵ PER = g gain / g protein fed.

⁶ PPV = retained protein (g) / protein fed (g).

The highest condition factor (CF) was recorded in fish fed the FM40+SM diet and was significantly higher than the FM40+SM+KM and FM40+PM treatments ($p < 0.05$), but none of these three treatments showed significant differences from fish fed FM100 diet ($p > 0.05$). Fish fed diets with FM40+PM and FM40+PM+KM had the lowest hepatosomatic

index among all the treatments ($p < 0.05$). Intraperitoneal fat (IPF) ratio of fish fed the FM40+SM+FS diet or FM40+PM+FS diet were significantly higher compared to other experiment groups with the exception of the FM40+PM+KM group. (Table 3)

Table 3. Morphological indices of juvenile *L. argentimaculatus* fed experimental diets for 8 weeks¹

	CF ²	HSI ³	IPF ratio ⁴
FM100	1.88±0.07 ^{ab}	2.78±0.40 ^a	1.02±0.05 ^b
FM40+SM	1.96±0.01 ^a	2.95±0.21 ^a	1.61±0.15 ^b
FM40+SM+FS	1.88±0.12 ^{ab}	3.12±0.11 ^a	2.44±0.26 ^a
FM40+SM+KM	1.78±0.03 ^b	2.98±0.17 ^a	1.26±0.28 ^b
FM40+PM	1.76±0.03 ^b	1.73±0.33 ^b	1.13±0.31 ^b
FM40+PM+FS	1.89±0.10 ^{ab}	2.95±0.39 ^a	2.30±0.72 ^a
FM40+PM+KM	1.86±0.08 ^{ab}	2.14±0.25 ^b	2.54±0.40 ^a

¹ Values are means ±S.E.M. of three replicates and values within the same row with different letters are significantly different ($P < 0.05$).

² CF (condition factor) = $100 \times (\text{live weight, g}) / (\text{body length, cm})^3$.

³ Hepatosomatic index (HSI) = $\text{liver weight} \times 100 / \text{body weight}$.

⁴ Intraperitoneal fat (IPF) ratio = $\text{IPF weight} \times 100 / \text{body weight}$.

Moisture content value was significantly higher ($p < 0.05$) in the FM40+PM group when compared with other experiment groups but whole-body lipid content value was lower (Table 4). Fish fed the FM40+PM diet displayed significantly lower ($p < 0.05$) whole-body protein content than fish fed the FM40+SM diet, the FM40+SM+FS diet, FM40+SM+KM, FM40+PM+FS diet, or FM40+PM+KM diet. Plasma total protein (TP), cholesterol (CHOL), total triacylglycerol (TG) and high-density lipoprotein-cholesterol (HDL-C) for experimental fish groups were only slightly influenced by the different dietary treatments (Table 5). The low-density lipoprotein-cholesterol (LDL-C) value in the FM40+PM+FS group was significantly higher than that of LDL-C in the FM100 or FM40+SM+KM group ($p < 0.05$).

Table 4. Whole-body composition (fresh-weight basis) of juvenile *L. argentimaculatus* fed the experimental diets for 8 weeks¹

	Moisture (%)	Protein (%)	Lipid (%)
FM100	71.77±1.49 ^b	18.35±0.59 ^{cd}	5.88±0.59 ^b
FM40+SM	70.52±0.21 ^b	19.46±0.59 ^{ab}	8.49±0.16 ^a
FM40+SM+FS	70.22±1.67 ^b	20.11±0.54 ^a	6.54±0.70 ^b
FM40+SM+KM	71.57±0.32 ^b	19.24±0.34 ^{abc}	6.29±0.26 ^b
FM40+PM	73.99±0.82 ^a	17.56±1.00 ^d	4.25±0.69 ^c
FM40+PM+FS	71.50±0.24 ^b	18.98±0.31 ^{bc}	5.80±0.60 ^b
FM40+PM+KM	70.71±0.78 ^b	19.49±0.47 ^{ab}	6.77±0.40 ^b

¹ Values are means ±S.E.M. of three replicates and values within the same row with different letters are significantly different ($P < 0.05$).

Table 5. Plasma composition of juvenile *L. argentimaculatus* fed experimental diets for 8 weeks¹

	TP (g/L) ^a	TG (mmol/L) ^b	CHOL (mmol/L) ^c	HDL-C (mmol/L) ^d	LDL-C (mmol/L) ^e
FM100	41.73±8.1	4.57±0.5	4.47±0.9	2.13±0.4	0.46±0.3 ^c
FM40+SM	43.20±6.2	3.43±1.7	4.63±1.3	2.40±0.8	0.67±0.3 ^{abc}
FM40+SM+FS	41.47±3.6	3.03±0.4	4.00±0.2	2.13±0.1	0.49±0.1 ^{bc}
FM40+SM+KM	43.20±7.3	3.87±1.6	4.80±1.5	2.13±0.6	0.91±0.3 ^{ab}
FM40+PM	40.73±6.8	4.50±1.6	4.90±0.5	2.07±0.1	0.79±0.2 ^{abc}
FM40+PM+FS	37.07±1.6	3.07±0.6	4.17±0.2	2.03±0.2	0.74±0.2 ^{abc}
FM40+PM+KM	43.60±9.3	3.83±0.8	5.10±0.4	2.37±0.2	0.99±0.1 ^a

¹ Values are means ±S.E.M of three replicates and values within the same row with different letters are significantly different ($P < 0.05$).

^a TP: total protein;

^b TG: total triacylglycerol;

^c CHOL: cholesterol.

^d HDL-C: high density lipoprotein-cholesterol;

^e LDL-C: low density lipoprotein-cholesterol.

Discussion

The present study indicated that 60% of dietary FM protein can be replaced by SBM alone, or SBM combined with FS or KM. WG was 68%-76% for fish fed a diet with 40% crude protein, 9% lipid, and a 60% replacement of fishmeal by soybean meal or peanut meal. These were comparable to previous results reported for this fish species (Abbas and Siddiqui, 2013). 50% fishmeal protein could be replaced without significantly reducing growth of *Lutjanus argentimaculatus* fed diets containing 50% crude protein and 10% crude lipid (Catacutan and Pagador, 2004). In contrast with FM40+PM and FM40+PM+FS groups, which had the lowest WG among all the treatments, a mixture of peanut meal with 10% KM (FM40+PM+KM) obtained similar growth performance results to the control group. This indicated that 10% KM supplement had a positive influence on the growth of *Lutjanus argentimaculatus* when fish were fed peanut meal. However, fish growth performance was not further improved by KM supplementation when SBM was added to the diet. A previous study indicated that KM has been successfully used in diets for Atlantic salmon as the main protein source, but slightly reduced fish growth was observed when totally replacing FM (Hansen et al., 2010). Growth was promoted by adding FS in SB and FS supplemented diets even though no significant differences were found. These results are in accordance with the study on Atlantic salmon where FS had a stimulating effect on fish growth when fish were fed high plant protein diets (Kousoulaki et al., 2009).

Limited palatability of the high plant protein diet has been recognized as one of the major negative effects for marine fish. In this study, no significant differences in feed intake were observed among all the experiment treatments. One of the possible reasons could be that amino acid mix (Met, Lys, taurine and glycine) was added into the FM40+SM and FM40+PM groups as fish feed intake could be affected by the dietary amino acid profile (Webster et al., 1995). The addition of KM and FS have generally had attractive and palatable characteristics. KM may stimulate olfactory and gustatory responses (Shimizu et al., 1990) and has similar amino acid profile as FM. Feed intake is improvements with supplementation of KM have also been recorded in several fish species such as tilapia (Gaber, 2005), Atlantic cod and Atlantic halibut (Tibbetts et al., 2011). As a fish meal processing by-product, FS contain sorts of water soluble molecules and particles i.e., free amino acids, peptides and low molecular weight components such as taurine, creatinine, carnosine, etc. (Kousoulaki et al., 2009) which have been proven to be feeding stimulatory substances (Kader et al., 2010, 2012).

In this study, an obvious positive correlation was observed between weight gain (WG) and feed utilization (FE, PER, PPV). The lower feed utilization in fish fed the FM40+PM diet may be due to its reduced digestibility, nutrient imbalances, or anti-nutritional factors which need further study. In the study of Mozambique Tilapia, lower FE was also recorded in fish when fish meal was replaced with higher levels of peanut meal (Yildirim et al., 2014).

Higher values of IPF ratio and whole-body lipid content observed in the FM40+SM group indicated that replacement of fishmeal by soybean meal could induce lipid deposition in fish. These results may be attributed to the insufficiency of available phosphorus in high-plant stuff diets (Robaina et al., 1995). Values of hepatosomatic index in FM40+PM and FM40+PM+KM treatments were significantly lower than those of the fishmeal-based control treatment or other experimental treatments. The lowest values of whole-body protein and lipid content recorded in the FM40+PM group compared with other groups may be due to the lower digestibility and feed efficiency of high levels of PM as found in another study (Yildirim et al., 2014).

Biochemical parameters of plasma are useful to monitor the physiological status and nutrients metabolism in fish (Li et al., 2010). Plasma substrate concentrations are affected by many factors such as the transcript capability of intestine epidermal cells, efficiency of fish digestive system to absorb nutrients and the metabolism status of fish. In our study plasma TP, CHOL, TG and HDL-C were not remarkably influenced by different dietary treatments. These results may indicate that fish had a relatively good physiological response to the experimental diets and were held under healthy conditions.

In conclusion, 60% replacement of FM by soybean meal can be achieved in juvenile mangrove red snapper diets without any reduction in growth performance. On the other hand, the peanut meal substitution to FM at a 60% level significantly reduced fish growth performance in the absence of 10% FS or KM supplementation.

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